

Prenatal Pesticide and PCB Exposures and Birth Outcomes

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ABSTRACT: Evidence is inconsistent or poorly understood for links between polychlorinated biphenyls (PCBs), 1,1'-dichloro-2,2'-bis(4-chlorophenyl)ethylene (DDE), and organophosphate (OP) pesticides and adverse pregnancy outcomes, although they are known developmental toxicants. We measured biomarkers of maternal exposure to DDE, PCB, and OP metabolites in the third trimester of pregnancy among 404 mothers in a multiethnic cohort in New York City. We also determined maternal paraoxonase (PON1), butyrylcholinesterase (BuChe), and *PON1Q192R* gene variant. Higher multivariate-adjusted DDE levels (but not PCB) were associated with lower birth weight ($-98 \text{ g}/\log_{10} \text{ DDE}$, $p = 0.096$) and head circumference ($-0.54 \text{ cm}/\log_{10} \text{ DDE}$, $p = 0.030$). DDE and PCB levels were not related to birth length, Ponderal index, or gestational age. Birth length was shorter for mothers with *PON192RR* slow genotype compared with *PON192QQ* ($p = 0.026$), and head circumference was inversely associated with maternal PON1 activity ($p = 0.004$). With slow-activity PON1 or *PON192*, urinary diethylphosphates (ΣDEPs) were associated with lower birth weight and dimethylphosphates (ΣDMPs) with shorter birth length. No associations were found between birth outcomes and BuChe. In summary, we found suggestive relationships between prenatal environmental biomarkers and birth outcomes in this population. Maternal susceptibility factors including PON1 and maternal weight contributed to the observed effects. (*Pediatr Res* 61: 243–250, 2007)

There is widespread concern about potentially adverse health effects of environmental chemicals on children. Infants exposed *in utero* and during the early neonatal period are particularly vulnerable because of their rapid growth, cell differentiation, immaturity of metabolic pathways, and development of vital organ systems (1). Pesticides including persistent organochlorines (OCs) such as DDE and PCBs have been associated with detrimental effects on childhood neurobehavioral development (2), but evidence is inconsistent regarding their effects on birth outcomes. Furthermore, only recently have data on effects of nonpersistent pesticide exposure have begun to emerge (3–5). Because adverse birth outcomes may be intermediate between prenatal toxic exposures and child neurologic effects, the *in utero* effects of environmental agents on pregnancy outcomes are of interest.

OCs continue to be detected in women of childbearing age (6–8), although such chemicals were banned from commercial use in the 1970s. OPs have been widely used for decades and represent about half of insecticides used in the United States (9). Before 2000, approximately 60 million pounds were used annually in agriculture and another 17 million pounds for nonagricultural purposes, including spraying in residential buildings (9). OPs are highly neurotoxic, yet developmental effects in children have been little studied. Exposure has been documented for the general population including pregnant women and their children both in rural (10) and urban areas (4,11) as well as in agricultural populations (5).

The present investigation examines effects of *in utero* exposure to OPs and OCs on fetal growth in an urban cohort of mothers and their infants. We have previously reported results of effects on birth outcomes of exposure to 3,5,6-trichloro-2-pyridinol, a metabolite of the OP pesticide chlorpyrifos as well as 3-phenoxybenzoic acid, a pyrethroid metabolite, and pentachlorophenol, a wood preservative and a metabolite of hexachlorobenzene (3). In this report, we describe birth outcomes with respect to OP metabolites, PCBs, DDE, and lead measured in maternal samples obtained in the third trimester of pregnancy.

MATERIALS AND METHODS

The Children's Environmental Health Study is a prospective study that is following an ethnically diverse cohort of mother-infant pairs enrolled at Mount Sinai Hospital in New York City during pregnancy from March 1998 to March 2002. Details regarding study design and protocols have been described previously (3). Excluded were multiparas; multiple gestations; first prenatal visit after 26 wk of gestation; serious chronic diseases such as diabetes, hypertension, and thyroid disease; serious pregnancy complication; consuming more than two alcoholic beverages per day; and using illegal drugs. Of 479 recruited women, 75 were excluded because of medical complications, severe prematurity ($<1500 \text{ g}$ or $<32 \text{ wk}$ of gestation), infant birth defects, no biologic specimens collected before birth, change of residence outside New York City, or refusal to continue participation. The final sample size for this analysis was 404 births. This study was approved by the Institutional Review Board of Mount Sinai School of Medicine, and signed informed consent was obtained.

Abbreviations: BuChe, butyrylcholinesterase; DDE, 1,1'-dichloro-2,2'-bis(4-chlorophenyl)ethylene; DEDP, diethyldithiophosphate; DMDP, dimethyldithiophosphate; DMTP, dimethylthiophosphate; LOD, limit of detection; MDA, malathion dicarboxylic acid; OCs, organochlorines; OP, organophosphate; PCBs, polychlorinated biphenyls; PON1, paraoxonase; ΣDAPs , dialkylphosphates; ΣDEPs , diethylphosphates; ΣDMPs , dimethylphosphates

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Table 1. Blood and urine analytes in prenatal maternal specimens

	<i>n</i>	LOD	% >LOD	Median	Min	Max
Blood measures						
Blood lead, $\mu\text{g}/\text{dL}$	292	0.1	88.0	0.90	0.10	6.3
DDE, $\mu\text{g}/\text{L}$	194	0.07	97.4	0.64	0	57.3
PCBs, $\mu\text{g}/\text{L}$	194	0.3	94.3	0.79	0.14	7.3
DDE, ng/g lipid*	174	10	98.9	110	0	9240
PCBs, ng/g lipid*	174	50	94.8	150	20	1060
Urine measures						
ΣDEPs , nm/L†	327	‡	88.1	18.8	0	429
ΣDMPs , nm/L†	336	‡	92.6	42.2	0	4903
ΣDAPs , nm/L† ($\Sigma\text{DAP} = \Sigma\text{DMP} + \Sigma\text{DEP}$)	327	‡	97.2	75.9	0	4987
ΣDEPs , nm/g creatinine	327	‡	88.1	22.1	0	1002
ΣDMPs , nm/g creatinine	336	‡	92.6	55.4	0	2071
ΣDAPs , nm/g creatinine ($\Sigma\text{DAP} = \Sigma\text{DMP} + \Sigma\text{DEP}$)	327	‡	97.2	88.6	0	2106
MDA $\mu\text{g}/\text{L}$ †	361	0.3	20.5	LOD	LOD	15.8

* PCBs are the sum of four congeners, 118, 138, 153, and 180. LOD is approximate based on the LOD ($\mu\text{g}/\text{L}$)/mean total lipids (5.5 g/L); lipids were not available for 20 samples.

† Total number includes samples with creatinine ≥ 20 .

‡ Based on an individual analyte value equal to or greater than LOD for any of the three metabolites in each class (1–4 nm/L, 0.2–0.5 $\mu\text{g}/\text{L}$).

A questionnaire was administered to participants during their third trimester of pregnancy to obtain information on pesticide and other environmental exposures, sociodemographic characteristics, obstetric and medical history, and lifestyle factors. Information on delivery characteristics and birth outcomes, including birth weight, length, head circumference, gestational age, and infant gender were obtained from a computerized perinatal database within the Department of Obstetrics, Gynecology, and Reproductive Science at Mount Sinai Hospital. Measurements of weight, length, and head circumference at birth were based on standardized clinical techniques. Maternal blood and urine samples were obtained during the third trimester (generally between 26 and 28 wk). Cord blood samples were obtained at the time of birth. Maternal blood plasma was used to analyze DDE and PCB levels in a random subset of women ($n = 194$). Maternal urine samples collected before January 2001 ($n = 386$) were analyzed by the Centers for Disease Control and Prevention (CDC) for six alkylphosphate metabolites (APs) and malathion dicarboxylic acid (MDA). Lead levels (PbB) were also measured in cord blood ($n = 102$) or maternal whole blood ($n = 192$) if a cord blood sample was not available.

Laboratory and quality control methods have been reported previously (7,12–14). Limits of detection (LOD) based on three times the standard deviation (SD) of blanks run during the determinations are presented in Table 1. Laboratory values were available for diethylthiophosphate (DEDP) ($n = 344$; 7% >LOD), ΣDEP ($n = 316$; 45% >LOD), diethylthiophosphate ($n = 348$; 81% >LOD), dimethylthiophosphate (DMDP) ($n = 357$; 25% >LOD), dimethylphosphate (ΣDMP) ($n = 328$; 58% >LOD), dimethylthiophosphate (DMTP) ($n = 346$; 89% >LOD). Samples of urine with <20 mg/dL creatinine ($n = 27$) were excluded from metabolite analyses. Concentrations were imputed for missing values (5); metabolites were then summed (as nm/L) to obtain ΣDEP (DEDP, diethylphosphate, diethylthiophosphate), ΣDMP (DMDP, dimethylphosphate DMTP), and ΣDAP ($\Sigma\text{DEP} + \Sigma\text{DMP}$). PON1 activity was determined as previously reported in plasma ($\mu\text{mol}/\text{min}/\text{mL}$) using phenylacetate as substrate (15). BuChE activity ($\mu\text{mol}/\text{min}/\text{mL}$) was determined by the method of Ellman *et al.* (16) using 1 mmol/L butyrylthiocholine as substrate in 50 mmol/L phosphate buffer, pH 8.0, at 25°C, following absorbance at 412 nm. The assay within-batch precision was 3.2% for PON1 and 8.6% for BuChE. *PONQ192R* was genotyped ($n = 389$) as reported (17). This *PON1* variant was chosen because its maternal phenotypic variability exhibits a range of PON1 expression different from other *PON1* variants (15,18). PCBs were defined as the sum of congeners 118, 153, 138, and 180 (7). Total lipids (g/L) were calculated using cholesterol and triglycerides (19) determined on 174 plasma samples with sufficient volume.

Statistical analyses were performed using SAS-PC, version 9.1 (SAS, Inc., Cary, NC). Models predicting birth outcomes were fit using SAS General Linear Models Procedure (Proc GLM); covariates included environmental biomarker values, maternal race/ethnicity (white *versus* nonwhite), infant sex, and, except for those analyses predicting gestational age, gestational age. Models with ΣDAPs included *PON1* phenotype as tertiles or *PON192* genotype (*RR*, *RQ*, *QQ*), or BuChE. OC models were adjusted for maternal age. Additional covariates were identified using bivariate analyses for biomarkers (Spearman correlation for continuous and χ^2 or Mantel-Haenszel (M-H) for categorical variables with $p < 0.15$), including those reported previously for OCs (7). Race, education, and marital status were highly correlated, and

therefore only race was included in birth outcome models (3). Continuous biomarker values were log-transformed (\log_{10} to be comparable with references 5,20), and 0.5 was added to ΣDAPs to remove zero values. Potential covariates were added to the base model for each birth outcome, and backward stepwise elimination was used to remove covariates that altered biomarker betas by <15%. Final alkylphosphate models did not have entered any variables besides the base covariates. In OC models, the only covariate added to models was maternal pregnancy weight gain. However, OCs and birth outcomes are each known to be associated with both maternal weight gain and prepregnancy body mass index (BMI) (21–24). Therefore, to facilitate interpretation of the results, we present models adjusted for both variables and their interaction (using categorical variables for each, less than and equal to or greater than medians). The interaction term was not statistically significant, and using weight gain and BMI as continuous variables or quartiles did not change the DDE effect. Because pregnancy weight gain ($n = 16$) and lipids ($n = 20$) were missing for 194 women with DDE and PCB values, we constructed models in the subset of 178 with complete covariate data apart from lipids and report separately the models without and with lipid adjustment in the subset having all variables ($n = 160$). Using individual lipid-corrected values (*i.e.* ng/g lipid) did not change findings nor did adjusting for triglycerides and cholesterol instead of total lipids. Based on pharmacokinetics, the greatest OP exposure potential should exist if the slowest *PON1* activity (first tertile or *PON192RR* genotype) were combined with high exposure (equal to or greater than median ΣDAPs); the least potent subgroup would be fastest *PON1* activity (third tertile or *PON192 QQ* genotype) with low exposure potential (less than median ΣDAPs). Therefore, we compared least squares means for these extremes by examining the interactions between APs and *PON1* or *PON192* genotype for those models where a main effect at $p < 0.1$ had been seen for urinary APs, *PON1*, or *PON192*.

RESULTS

Maternal biomarker levels are presented in Table 1. Maternal plasma DDE and PCB levels both had median values <1 $\mu\text{g}/\text{L}$. Median OC and OP concentrations were 1–100 $\mu\text{g}/\text{L}$, except for MDA, the malathion-specific metabolite that was detectable in only 20% of samples tested. ΣDMP urinary metabolites were about threefold higher than ΣDEP in urine. PbB levels were very low and not related to any covariates or birth outcomes.

Levels of OCs (DDE and PCB) were higher in older women, in white women, in married women, and those with college education (Table 2), suggesting an age-related association with higher socioeconomic status. DDE was higher in leaner women and lower in participants recruited after January 2001.

Table 2. Biomarkers by maternal characteristics of the study population ($n = 404$)

Characteristics	DDE, $\mu\text{g/L}$, median (n)	PCB, $\mu\text{g/L}$, median (n)	DDE, ng/g lip, median (n)	PCB, ng/g lip, median (n)	ΣDEP , nm/L, median (n)	ΣDMP , nm/L, median (n)	ΣDAP , nm/L, median (n)	MDA, % >LOD (n)
Maternal age, y								
<20 ($n = 142$)	0.41 (57)	0.63 (57)	80 (53)	140 (53)	14.7 (123)	33.7 (127)	56.7 (123)	13.9 (18)
20–24 ($n = 132$)	0.54 (59)	0.64 (59)	100 (56)	130 (56)	19.9 (118)	35.1 (119)	75.7 (118)	20.8 (25)
25–29 ($n = 44$)	1.11 (27)	0.93 (27)	150 (24)	170 (24)	12.8 (37)	60.4 (40)	90.4 (37)	17.5 (7)
30–34 ($n = 64$)	1.16 (38)	0.99 (38)	210 (30)	220 (30)	24.1 (49)	63.4 (51)	92.7 (49)	35.3 (18)
35+ ($n = 22$)	1.25 (13)†	1.84 (13)†	200 (11)†	310 (11)†	10.2 (21)	54.0 (21)	85.5 (21)	28.6 (6)†
Race/ethnicity								
Black ($n = 112$)	0.47 (66)	0.73 (66)	100 (62)	160 (62)	15.6 (92)	47.3 (94)	78.9 (92)	22.1 (21)
Hispanic ($n = 200$)	0.48 (64)	0.66 (64)	90 (61)	120 (61)	19.1 (182)	32.3 (187)	63.3 (182)	18.0 (34)
White ($n = 86$)	1.16 (64)†	1.13 (64)†	180 (51)†	220 (51)†	20.8 (68)	70.0 (71)†	92.9 (68)	25.3 (18)
Other ($n = 6$)	—	—	—	—	6.94 (6)	25.7 (6)	30.7 (6)	16.7 (1)
Marital status								
Married ($n = 117$)	1.02 (74)	0.96 (74)	180 (61)	200 (61)	19.6 (97)	58.6 (100)	88.1 (97)	28.7 (29)
Living with baby's father ($n = 98$)	0.47 (39)	0.75 (39)	90 (36)	140 (36)	13.0 (87)	27.5 (90)	48.0 (87)	17.6 (16)
Divorced/widowed/separated/single ($n = 189$)	0.45 (81)†	0.70 (81)†	90 (77)†	140 (77)†	22.3 (164)	42.4 (168)*	82.8 (164)*	17.2 (29)*
Education								
<HS ($n = 118$)	0.45 (44)	0.63 (44)	90 (40)	130 (40)	14.7 (101)	41.8 (106)	80.3 (101)	18.5 (20)
HS grad ($n = 83$)	0.47 (38)	0.78 (38)	90 (37)	140 (37)	15.6 (75)	35.3 (75)	80.6 (75)	17.3 (13)
Some college ($n = 103$)	0.56 (46)	0.65 (46)	110 (44)	140 (44)	19.1 (92)	31.1 (94)	57.7 (92)	21.0 (20)
Bachelor's or higher ($n = 98$)	1.15 (65)†	1.16 (65)†	190 (53)†	220 (53)†	23.2 (79)	69.6 (82)	91.0 (79)	25.6 (21)
Pregnancy BMI								
<20 ($n = 82$)	0.76 (37)	0.90 (37)	120 (35)	140 (35)	13.4 (70)	47.8 (73)	83.3 (70)	12.3 (9)
20–24.9 ($n = 214$)	0.74 (106)	0.89 (106)	130 (93)	180 (93)	22.6 (185)	36.7 (191)	77.3 (185)	21.2 (41)
25–29.9 ($n = 72$)	0.42 (30)	0.64 (30)	90 (27)	140 (27)	15.5 (64)	35.8 (64)	62.1 (64)	23.4 (15)
30+ ($n = 35$)	0.48 (21)†	0.62 (21)†	100 (19)*	140 (19)	13.7 (29)	54.4 (30)	87.2 (29)	29.0 (9)†
Smoking								
No ($n = 383$)	0.69 (181)	0.82 (181)	120 (162)	150 (162)	16.5 (329)	42.2 (338)	75.8 (329)	20.5 (70)
Yes ($n = 21$)	0.45 (13)†	0.71 (13)	100 (12)	170 (12)	29.1 (19)	53.0 (20)	80.6 (19)	20.0 (4)
Year of blood specimen collection								
Before 2001 ($n = 370$)	0.65 (175)	0.80 (175)	120 (162)	160 (162)	17.7 (338)	43.1 (348)	75.9 (338)	20.8 (73)
PON tertile (Maternal Plasma)								
1 (<96 mg/m · mL, $n = 130$)	0.57 (65)	0.76 (65)	130 (61)	180 (61)	13.5 (119)	33.8 (120)	67.3 (119)	15.8 (19)
2 (97–116.6, $n = 123$)	0.75 (57)	0.93 (57)	130 (56)	150 (56)	16.4 (108)	40.1 (111)	69.3 (108)	20.5 (23)
3 (116.7–200, $n = 130$)	0.56 (60)	0.74 (60)	100 (57)	140 (57)*	19.9 (111)	51.3 (117)	83.5 (111)	24.4 (29)
After Jan. 1, 2001 ($n = 34$)	0.44 (19)*	0.64 (19)	80 (12)*	100 (12)	22.8 (10)	26.7 (10)	60.0 (10)	10 (1)
1 (<96 mg/m · mL, $n = 130$)	0.57 (65)	0.76 (65)	130 (61)	180 (61)	13.5 (119)	33.8 (120)	67.3 (119)	15.8 (19)
2 (97–116.6, $n = 123$)	0.75 (57)	0.93 (57)	130 (56)	150 (56)	16.4 (108)	40.1 (111)	69.3 (108)	20.5 (23)
3 (116.7–200, $n = 130$)	0.56 (60)	0.74 (60)	100 (57)	140 (57)*	19.9 (111)	51.3 (117)	83.5 (111)	24.4 (29)
BuChe tertile (maternal plasma)								
1 ($n = 125$) <1.9	0.74 (63)	0.86 (63)	130 (59)	180 (59)	12.2 (101)	35.4 (104)	63.1 (101)	17.9 (19)
2 ($n = 124$) 1.9–2.43	0.49 (63)	0.70 (63)	100 (60)	140 (60)	24.6 (108)	67.2 (112)	101.1 (108)	19.5 (22)
3 ($n = 124$) 2.44–13.8	0.64 (56)	0.79 (56)	110 (51)	150 (51)	20.9 (113)	34.1 (116)	58.1 (113)	23.3 (27)
Maternal PON1/IQ192 genotype								
3 ($n = 95$) RR (low activity variant)	0.51 (42)	0.79 (42)	110 (41)	150 (41)	22.6 (81)	55.6 (83)	90.4 (81)	24.4 (21)
2 ($n = 174$) RQ	0.57 (86)	0.72 (86)	110 (81)	150 (81)	14.0 (158)	34.4 (163)	64.3 (158)	12.9 (21)
1 ($n = 120$) QQ	0.81 (58)	0.89 (58)	130 (52)	190 (52)	25.6 (100)	47.8 (103)	82.6 (100)	31.1 (32)†

Thirteen persons with DDE/PCB did not have AP urine analyses done. These 13 DDE/PCB samples were collected after January 2001. No urine samples collected after January 2001 were analyzed. ΣDAP is the sum of ΣDMP and ΣDEP . Other group was excluded from racial/ethnic comparisons.

* $p < 0.10$ or † $p < 0.05$ for Spearman, Kruskal-Wallis, or χ^2 statistics.

ing both PCBs and DDE in birth outcome models did not change effects of either compound.

The pronounced joint effect of both DDE levels and maternal weight gain on birth weight and head circumference prompted us to further explore these relationships while accounting for maternal prepregnancy BMI (Fig. 2). Lower maternal weight gain/BMI accompanied smaller birth weight or head circumference, higher DDE levels, and older maternal age. Thus, from high to low extremes of weight gain/BMI, birth weight decreased by -270 g (crude or adjusted ± 84 SE, $p = 0.0014$, $n = 178$) and head circumference decreased by 0.4 – 0.5 cm (crude or adjusted: ± 0.4 SE, $p = 0.18$). Over the same extremes, DDE increased from 0.48 to 1.4 $\mu\text{g/L}$ (unadjusted geometric means) and mean maternal age from 23 to 28 y (Fig. 2). Therefore, DDE birth outcome models may have residual confounding due to our inability to disentangle these effects.

Maternal ΣDAPs exhibited weak, nonsignificant inverse associations with three birth outcomes: ΣDEP with birth weight, ΣDEP with Ponderal index, and ΣDAP with head circumference (adjusted; Table 3). MDA was not associated with any birth outcomes ($p > 0.15$). Excluding or adjusting for mothers recruited after January 2001 did not change associations of APs with birth outcomes, although there were few such women in our sample. Creatinine adjustment made little further change in the estimates possibly because we

had excluded the most dilute urine samples (<20 mg/dL creatinine).

As reported previously (3), a strong association was observed between maternal PON1 activity and head circumference (Table 4). In models using tertiles instead of continuous PON1, head circumference was 0.62 ± 0.18 cm smaller in the first (slow activity) compared with the third (fast activity) tertile of PON1 (adjusted, $n = 382$; $p = 0.0009$; not shown). Similarly, head circumference was 0.37 ± 0.21 cm shorter for *PON192RR* (slow/slow allele type, $n = 95$) versus *PON192QQ* (fast/fast, $n = 120$); $p = 0.082$, adjusted means; data not shown. Birth length was shorter by 0.68 ± 0.30 cm among mothers with *PON192RR* than *PON192QQ* allele type ($p = 0.026$, data not shown). The range of enzyme activities was similar for PON1 and BuChE (a ratio of 1.2 – 1.3 between the tertile cut points), but no associations were observed between exposures or birth outcomes with maternal BuChE.

Based on finding a main effect with APs ($p < 0.1$), we examined their interactions with PON1 and *PON192* for birth weight (ΣDEP), head circumference (ΣDAP), and Ponderal index (ΣDEP). For birth length, which was associated with *PON192*, we examined interactions with ΣDMP , ΣDEP , and ΣDAP . This approach is consistent with the metabolic pathway of OP (Fig. 2) and our previous finding of an interaction between PON1 and 3,5,6- trichloropyridinol (3). For birth

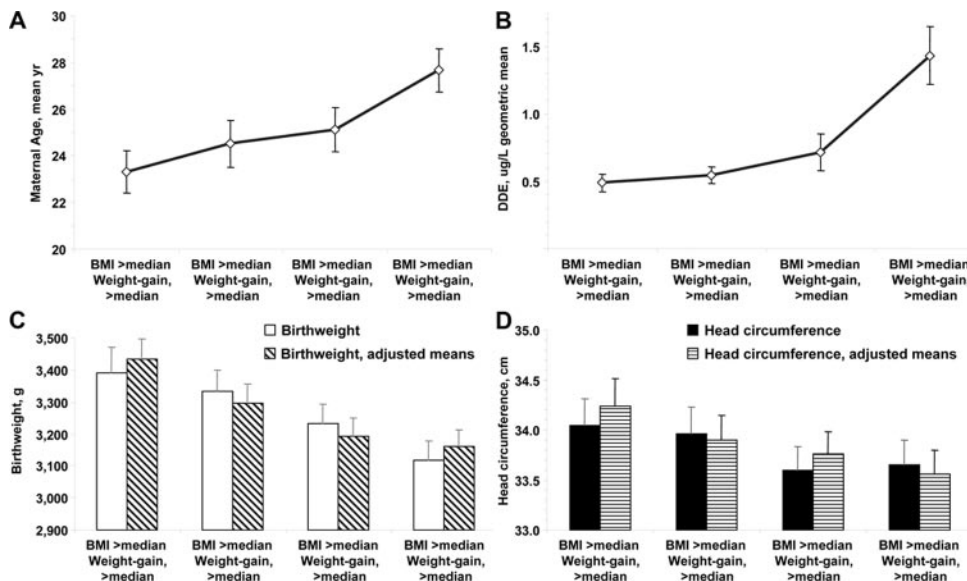


Figure 2. (A, B) The plots show mean maternal age and geometric means of DDE by maternal pregnancy weight gain/BMI categories. (C, D) The bar graphs show birth weight and head circumference means by weight gain/BMI, crude and adjusted for \log_{10} DDE, maternal age, race/ethnicity, sex, and gestational age (models in Table 3). Error bars are SE.

Table 4. Regression coefficients (\pm SE) of birth outcomes in relation to maternal PON1 and BuChE enzymatic activity, Children's Environmental Health Study, Mount Sinai Hospital, 1998–2002

Birth outcome	PON1 enzymatic activity (mg/mL \cdot min)		BuChE enzymatic activity (mg/mL \cdot min)	
	($\beta \cdot 10^3 \pm \text{SE} \cdot 10^3$)	<i>p</i>	$\beta \pm \text{SE}$	<i>p</i>
Birth weight,* g	8.88 ± 7.32	0.226	31.8 ± 330	0.923
Birth length,* cm	-0.004 ± 0.04	0.920	0.25 ± 1.83	0.889
Head circumference,* cm	0.081 ± 0.028	0.004	0.44 ± 1.27	0.728
Gestational age,† wk	-0.033 ± 0.029	0.254	-0.45 ± 1.32	0.732
Ponderal Index*	0.007 ± 0.005	0.135	0.024 ± 0.23	0.916

The PON β values are presented after being multiplied by 10^3 .

* Adjusted for sex, race, and gestational age.

† Adjusted for sex and race.

Table 5. Adjusted means \pm SE of birth weight and length in relation to interactions between maternal urinary prenatal Σ DAP levels, *PON1*92 genotype, and *PON1* activity, Children's Environmental Health Study, Mount Sinai Hospital, 1998–2002

Σ DEP and birth weight					
Maternal <i>PON1</i> tertile*	Low maternal Σ DEP < median level		High maternal Σ DEP \geq median level		<i>p</i> value for Δ -birth weight by Σ DEP within <i>PON1</i>
	<i>n</i>	Birth weight, g, mean \pm SE	<i>n</i>	Birth weight, g, mean \pm SE	
1st (slow)	60	3305 \pm 53	53	3233 \pm 56†	0.323
2nd	53	3348 \pm 57	51	3282 \pm 57	0.392
3rd (fast)	45	3396 \pm 64†	56	3279 \pm 54	0.138
Maternal <i>PON1</i> 92**	Low maternal Σ DEP < median level		High maternal Σ DEP \geq median level		<i>p</i> value for Δ -birth weight by Σ DEP within genotype
	<i>n</i>	Birth weight, g, mean \pm SE	<i>n</i>	Birth weight, g, mean \pm SE	
<i>RR</i> (slow)	39	3346 \pm 69	55	3254 \pm 63‡	0.291
<i>RQ</i>	84	3278 \pm 46	66	3285 \pm 50	0.907
<i>QQ</i> (fast)	33	3453 \pm 60‡	42	3232 \pm 52	0.005
Σ DMP and birth length					
Maternal <i>PON1</i> tertile§	Low maternal Σ DMP < median level		High maternal Σ DMP \geq median level		<i>p</i> value for Δ length by Σ DMP within <i>PON1</i>
	<i>n</i>	Birth length, cm, mean \pm SE	<i>n</i>	Birth length, cm, mean \pm SE	
1st (slow)	61	51.1 \pm 0.3	52	50.2 \pm 0.3	0.032
2nd	55	50.3 \pm 0.3	51	50.7 \pm 0.3	0.258
3rd (fast)	49	50.4 \pm 0.3	58	50.8 \pm 0.3	0.418
Maternal <i>PON1</i> 92¶	Low Σ DMP < median level		High Σ DMP \geq median level		<i>p</i> value for Δ length by Σ DMP within genotype
	<i>n</i>	Birth length, cm, mean \pm SE	<i>n</i>	Birth length, cm, mean \pm SE	
<i>RR</i> (slow)	43	50.6 \pm 0.4	53	49.9 \pm 0.3‡‡	0.164
<i>RQ</i>	85	50.4 \pm 0.3	70	50.7 \pm 0.3	0.158
<i>QQ</i> (fast)	35	51.0 \pm 0.3‡‡	41	50.8 \pm 0.3	0.695

* *p* value for the interaction term in the model = 0.878.

† *p* value for extremes (third *PON1*/low Σ DEP vs first *PON1*/high Σ DEP, 164 g) = 0.042.

** *p* value for the interaction term in the model = 0.0755.

‡ *p* value for extremes (high *PONQQ*/low Σ DEP vs low activity *PONRR*/high Σ DEP 199 g) = 0.020.

§ *p* value for the interaction term in the model = 0.036.

|| *p* value for extremes (high *PON1*/low Σ DMP vs low activity *PON1*/high Σ DMP, 0.3 cm) = 0.549.

¶ *p* value for the interaction term in the model = 0.230.

‡‡ *p* value for highlighted extremes (high *PONQQ*/low Σ DMP vs low activity *PONRR*/high Σ DMP, 1.0 cm) = 0.019.

In the Σ DMP-greater-than-the-median group, length was shorter among the *RR* genotype than the *QR* (*p* = 0.042) or *QQ* (*p* = 0.041) genotypes.

The first tertile of *PON1* and the *RR PON1*92 genotype are the slower activity groups.

Means adjusted for race, sex, gestational age, and creatinine level. Urinary metabolites included samples with creatinine >20 mg/dL. Cutpoints for the *PON1* second tertile were >96 to <116.7 μ g/mL/min.

weight, interaction terms were not significant for *PON1*- Σ DEP (*p* = 0.878) or *PON1*92- Σ DEP (*p* = 0.076) (Table 5). However, there was a 164-g deficit in birth weight between extremes of the interaction (*p* = 0.042), *i.e.* fast activity-*PON1* genotype/low Σ DEP (least risk) versus slow-*PON1*/high Σ DEP (greatest risk). A similar effect between extremes was seen for the *PON1*92 * Σ DEP interaction (−199, *p* = 0.020; Table 5). In addition, within only the fast *PON1*92*QQ* genotype, low Σ DEP * *PON1*92*QQ* birth weight was higher than high Σ DEP * *PON1*92*QQ* (*p* = 0.005; Table 5). For birth length, the interaction was significant between Σ DMP and *PON1* but not *PON1*92, yet effects were similar. Here, an exposure effect on birth length was seen within the slow activity *PON1* tertile (first). Thus, mothers with slowest *PON1* and high Σ DMP had shorter babies (−0.9 cm) than low Σ DMP (*p* = 0.032; Table 5). The effect was similar but not significant with slow *PON1*92*RR* (−0.6 cm in high Σ DMP versus low Σ DMP, *p* = 0.164). Moreover, birth length was significantly different between extremes of *PON1*92 genotype

with Σ DMP (−1.0 cm, *p* = 0.019). Exposure did not modify the effect of *PON1* or *PON1*92 to reduce head circumference, nor were there any significant effects of alkylphosphates on the Ponderal index when interactions with *PON1* were considered (data not shown).

DISCUSSION

Biomarker levels of DDE and PCBs, while low, are similar to those in other recent reports concerning women this age (6,8,25). Our MDA and Σ DMP levels were higher than the recent CDC report (5% \geq LOD MDA, 20 nm/L Σ DMP) but were half the levels reported among women working in agriculture in California (5). Σ DEP levels in this population were similar to those in the National Health and Nutrition Examination Survey and in California women (10 nm/L). Σ DMP levels were three times higher than Σ DEP metabolites in our study, in the California study (5), and in national data for U.S. adult women in 1999–2000 (25). Both DDE and AP levels

were lower among women recruited in later years (not age adjusted), as reported for OP pesticides in another New York City cohort of pregnant women (4). The strongest relationship among OP metabolites was MDA with DMDP, a primary malathion metabolite that is not produced *via* PON1. Substrates for both PON1 and BuChE are metabolites created by phase I type P-450 enzymes (Fig. 2), and therefore measurement of P-450 enzyme variants may be useful in addition to PON1 for assessing individual susceptibility to some OPs (Fig. 2). We did not measure these or other enzymes such as glucuronidase or sulfatase or additional OP-specific metabolites that might be formed through these pathways.

We observed smaller birth weight and head circumference in relation to higher maternal biomarker levels of DDE, although estimates were not statistically significant for birth weight. Positive weight gain and high maternal prepregnancy BMI are known to increase both birth weight and serum triglycerides (21) and also to decrease DDE (22,23) and PCB (24) levels. Therefore, the combined effect of maternal weight factors, DDE, and maternal lipids on birth outcomes is likely to be complex, and residual or unmeasured confounding among these variables may account for the observed associations, possibly further destabilized by our small sample size. For DDE, the effect size was in the range of 100-g birth weight and 0.5-cm head circumference accompanying a 10-fold change in DDE levels (1 \log_{10} unit). In addition, our exposure levels are even lower than other reports that did not find an effect of DDE on birth weight. Moreover, recent comprehensive reviews of this literature suggest that DDE effects on pregnancy, if any, are more likely to be related to pregnancy loss or preterm birth than to birth size (26,27). Another possibility is that the DDE association with birth weight is related to unmeasured lipids, such as fatty acids, that covary with OCs (28). Adjustment for serum lipids did not change our DDE estimates as much as maternal pregnancy weight gain.

Birth weight was marginally affected by OP metabolite levels of Σ DEP (<60 g lower birth weight per \log_{10} Σ DEP). Birth length was affected by Σ DMP, but only if maternal PON1 was considered. A small effect of Σ DAP on head circumference was no longer present when the interaction of Σ DAP with PON1 was taken into account. In the California pregnancy study, inverse relationships were seen between Σ DMP and gestational age (5) but not with weight, length, Ponderal index, or head circumference. Σ DEP levels were lower than Σ DMP levels in our study, yet we saw an effect of Σ DEP but not Σ DMP on birth weight. It is possible that effects of Σ DEP, if any, might be due to greater toxicity of the parent compounds or to different relative metabolism rates of OPs and their metabolites. Our findings are consistent with those in a minority population in northern Manhattan in which chlorpyrifos levels in cord plasma were associated with reduced birth weight and length (4). Σ DEP includes nonspecific urinary metabolites of chlorpyrifos, but the proportionality between cord plasma and maternal urinary levels is not known.

Of interest, among several reported relationships of low-level exposures with birth outcomes, the effect sizes are similar (*e.g.* -52 g in birth weight for 1 \log_{10} unit change in Σ DEP [-22 g per \log_e Σ DEP] in this study *versus* -43 g for

a \log_e unit change in blood chlorpyrifos in Whyatt *et al.* (4) and are <100 g/ \log_e biomarker, similar in magnitude to studies having nonsignificant associations of DDE with birth weight (20,29). In contrast, a larger and significant effect was found in a more highly exposed fish-eating population [-146 g birth weight/ \ln DDE (30)]. Finally, even a 200-g change in birth weight is unlikely to be clinically significant in a population of healthy infants such as ours, but it might be important among low birth weight infants. However, a 200 g-reduction is similar to that for smoking seven to eight cigarettes per day in the third trimester (31).

The influence of OPs on birth weight was stronger if individual susceptibility was taken into account. Individuals with lower PON1 activity are likely to have longer elimination rates and therefore higher OP levels in the body. Both phenotype and genotype are useful measures of susceptibility. Phenotype shows actual activity during pregnancy, but it is variable at any one time point (32), having weak associations with genotypes (15). In contrast, genotype may be considered an integrated indicator of PON1 activity; indeed, our suggestive trend of increasing birth weight with higher activity PON1 or *PON192* in low Σ DEP-exposed mothers is consistent with a previous report (33). The *PONQ192R* effect on head circumference is consistent with our previous report of lower PON1 and smaller head circumference (3). Here we see possible interactions of PON1 or *PON192* and Σ DEP levels with birth weight or Σ DMP with birth length. These observations may explain some differences between our study and the California results; the two studies also had a different racial/ethnic make up, which would alter PON1 genotype distribution and phenotype levels. Neither we nor the California study found an effect of BuChE on birth outcomes.

A strength of our study is the inclusion of prenatal exposure measurements in primiparous women. Our OC birth outcome findings may also represent a false-positive due to small numbers, as generally no effects have been observed even with quite large sample sizes (26,27). A limitation of some biomarkers is potential misclassification of exposure reflected in a single measurement; this is especially true for urinary Σ DAPs, which may not be highly reproducible between trimesters of pregnancy (10). The potential for coexposures to preformed OP metabolites in the environment exists, so these exposures may contribute to urinary Σ DAPs levels. Although OC body burden (total amount of OC) is likely to change little during pregnancy (no elimination), measured concentrations can be reduced due to dilution by increased maternal weight. Therefore, our findings and those of similar studies between birth weight and pesticide biomarkers may reflect maternal factors other than chemical exposure, and it is possible that differences across studies can be clarified with better knowledge of total exposure and individual pharmacokinetic factors, including metabolic variation.

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